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Combinations of Allelopathic Crop Extracts Reduce Digitaria spp. and Setaria faberi Seed Germination

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Abstract

Allelopathic cover crops contain compounds that deter other types of plant seeds from germinating or inhibiting established plants' growth. Sunflower (*Helianthus annuus*, SF), buckwheat (*Fagopyrum esculentum* Moench, BW), sorghum-sudangrass (*Sorghum* × *drummondii* [Nees ex. Steud.] Millsp. & Chase, SSG), and winter rye (*Secale cereale*) are all known allelopathic cover crops. However, there is little information about the use of these allelopathic cover crops used together and their combined impact on weed seed germination. Laboratory bioassays were conducted to determine the effect of the aforementioned cover crops alone and in combinations in reducing the germination rate of *Digitaria* spp. (crabgrass) and *Setaria faberi* (giant foxtail) through extract application. Two separate experiments were arranged as a 7 treatment × 3 extract rate factorial set out in a completely random design with three replicates. The first experiment used winter rye, sunflower, and sorghum-sudangrass with *Digitaria* spp., and the second experiment used sunflower, sorghum-sudangrass, and buckwheat with *S. faberi*. The 7 treatments were extracts of each cover crop species alone and in various binary and tertiary combinations. Each extract was applied at three concentrations: 3, 4, and 5% (g/v) extract. A water control was included. Winter rye alone or in combination with sunflower resulted in the lowest *Digitaria* spp. seed germination at extract concentrations 4% and 5%.

The 5% sorghum-sudangrass extract caused the greatest reduction in the number of *S. faberi* seeds germinated and the greatest reduction in the rate at which they germinated. This is congruent with the fact that extracts used individually were more effective than the control at reducing total and the rate of germination. In addition, binary combinations were also more effective than the control in reducing germination rate. The data that binary combinations are

more effective at reducing *S. faberi* germination than the control suggest a synergistic effect by various extracts used together at certain concentrations. This indicates that some of these cover crops may have potential value being used together in cover crop mixes to reduce *Digitaria* spp. and *S. faberi* weed pressure.

Introduction

All plants compete with one another for resources such as light, water, and nutrients. However, not all plants interfere with one another. In the science of allelopathy, interactions between allelopathic plants are studied, specifically those that exude substances that tend to inhibit the growth of other plant species (Zimdahl, 2013). For plants that are allelopathic, their allelochemicals can be isolated from all tissues. However, they tend to be more concentrated in some parts compared to others. For example, the leaves and roots tend to have higher concentrations than the flowers and fruits, and seeds tend to have the highest concentrations of these unique compounds. It is important to note that in some cases allelochemicals may stimulate the growth of other plant organisms (Putnam and Tang, 1986); however, we will only be discussing inhibitory effects of allelochemicals in this study. Allelochemicals may enter the soil system via root exudation, leaching from necrotic or living plant tissue, or, also, from volatilization from the shoots (Jabran et al., 2015). The allelopathic inhibition may come in the form of arresting weed development, stopping weed seeds from germinating, or delaying the weed seed germination.

Sometimes referred to as “plant herbicides”, plant allelochemicals could mitigate the farmer’s reliance on costly synthetic herbicides. According to Kadioglu and Yanar (2004), losses caused by weeds can be up to 24% of a farmer’s crop. This surpasses the potential threat posed

by disease causing pathogens and the damage wrought by pests, which are 16.4% and 11.2% respectively. The potential of allelopathic plant species may depend on cultivar. For example, when 99 rice cultivars were analyzed for their allelopathic potential, five reduced weed seed germination (%) and growth by over 50% (Mulbeen et al., 2012). These five were found to have higher concentrations of allelochemicals in their tissues compared to the other 94.

According to a review on the use of sorghum (*Sorghum* spp.) allelopathy in agroecosystems, farmers in Pakistan use an aqueous extract of sorghum spp. Residue commonly called sorgaab (Alsaadawi and Dayan, 2009). This water extract is sprayed on fields to decrease weed density and overall weed biomass.

Modes of action of allelochemicals differ from how many herbicides function, so the weeds would be less likely to be resistant to these compounds. In fact, many of the mechanisms affecting the target plant tissue include inhibiting aspects of amino acid synthesis, pigment synthesis, plasma membrane operation, and more (Vyvyan, 2010).

In this study, oilseed sunflower, winter rye, sorghum-sudangrass, and buckwheat a were used. Sunflower is known to interfere with the development of morning glory, velvetleaf, pigweed, jimsonweed, wild mustard, and others (Vyvyan, 2010). A major phenolic group of sunflower allelochemicals is the heliannuol family. This group of compounds tend to inhibit dicot plants.

Sorghum-sudangrass produces several allelochemicals such as cyanogenic glycoside (dhurrin), hydrophobic *p*-benzoquinone (sorgolone), courmaric acid, and ferrulic acid. These have been isolated from the shoots, roots, and from root exudates (Weston et al., 2013). Sorgolone is the most significant in terms of activity and content, and is continually released

from the root hairs as soon as the plant develops them. This allelochemical inhibits photosynthetic pathways and mitochondrial electron transport. In addition, it impacts an enzyme involved in the manufacturing of plastoquinone and also root H+ATPase activity. The study found that all cultivars being studied produced significant amounts of the allelochemical.

Although sixteen allelochemicals have been identified in *Secale cereale*, the major allelochemical of rye is DIBOA (2,4-dihydroxy-2H-1,4-benzoxazin-3(4H)-one), which degrades into BOA (benzoxazolin-2(3H)-one). Yet, both can be present in the same system simultaneously (Jabran et al. 2015; Burgos and Talbert 2000). These benzoxazolins are toxins to mostly small seeded weeds or species that do not have the capacity to effectively detoxify the allelochemicals (Macias et al. 2004). The effect on small seeds may be as result of the higher surface area to volume ratio. The mode of action has not been completely elucidated; however, it may relate to crippling effects on photophosphorylation, electron transport, cell differentiation, and root system of an affected plant (Albuquerque et al. 2010).

Several potentially allelopathic compounds have been isolated and identified in various cultivars of buckwheat. For example, Iqbal et al. (2002) showed that biologically active alkaloids found in buckwheat shoot tissues include fagomine, 4-piperidone, and 2-piperidinemethanol. Chloroform extracts of these respective alkaloids caused an 80% reduction in radicle elongation in lettuce seedlings when the concentration of the extracts was 100ppm or less. The same authors did a follow up study the subsequent year to further study buckwheat allelopathy. They found that gallic acid and (+)-catechin seem to exhibit allelopathic activity in that they inhibit the growth of various types of plants. Using aqueous extracts, the authors showed the EC_{50}[1] for

[1] EC_{50} refers to the estimated concentration of aqueous extract that causes 50% inhibition of shoot and root elongation

alfalfa (*Medicago sativa* L.), yellow mustard (*Brassica juncea* Czesn. Et Cross), white clover (*Trifolium repens*), and lettuce (*Lactuca sativa*) was at very low concentrations, 5-10µg ml^{-1}. (+)-catechin did not show a strong inhibitory effect on lettuce, but it did strain the growth of Indian mustard and Welsh onions. In addition, (+)-catechin inhibited the root and shoot growth of common amaranth and Italian ryegrass (*Lolium multiflorum* Lam., but at higher concentrations (50 and 100µg ml^{-1}) (Iqbal et al., 2003). Golisz et al. (2007) identifies another allelopathic buckwheat compound called rutin. This glycoside had the most severe effects on elongation of lettuce seedling root growth, and appears to be most responsible for allelopathic pressure from the Polish cultivar of buckwheat used.

The use of cover crops usually refers to the utilization of a plant that can have beneficial impacts on the soil (Dabney et al., 2001). For example, some cover crops can be used for reducing erosion between seasons, adding organic matter, increasing nitrogen levels, and other benefits as well. In fact, different cover crops can be used together in combination with one another in a seed mix to gain a variety of benefits. This, however, is not a practice typically associated with allelopathic cover crops, which can also offer benefits to soil quality as well as weed suppression (Jabran et al., 2015). Sunflower, sorghum-sudangrass, buckwheat, and winter rye have been reported to be allelopathic (Ahmad et al., 2000; Barnes et al., 1986; Bogatek et al,. 2006; Zimdahl, 2013). There is little research to suggest that these allelopathic cover crops have synergistic effects on weed suppression. Therefore, the objective of these experiments was to determine if there is a synergistic allelopathic effect on *Digitaria* spp. and *S. faberi* delivered by sunflower, sorghum-sudangrass, winter rye and buckwheat tissue extracts at different concentrations using laboratory bioassays. This study is divided into two experiments. In experiment 1, sunflower, winter rye, and sorghum sudangrass extracts were applied to *Digitaria*

spp. seeds. In experiment 2, sunflower, buckwheat, and sorghum sudangrass extracts were applied to *Setaria faberi* seeds.

***Experiment 1*: Use of sunflower, sorghum-sudangrass, and winter rye allelopathic extracts in laboratory bioassays**

Methods and Materials

Tissue preparation

Sunflower (common oilseed), sorghum-sudangrass, and winter rye seeds, whose cultivars were not specified, were obtained and sowed into pots size 900 with 3B SunGro soilless media on 13 February 2017. Cover crops grown were placed under natural light conditions, provided daily watering, and received one application of fertilizer on 12 March 2017. All plants received an application of 20-10-20 at 100 ppm N and Peter's S.T.E.M. (Soluble Trace Element Mix). The micronutrient blend provided sulfur (2.10 ppm), boron (0.20 ppm), copper (0.48 ppm), iron (1.13 ppm), manganese (1.2 ppm), and molybdenum (0.006 ppm). Plants received no supplemental lighting. The temperature setpoints in the greenhouse were 18.3˚C and 23.8˚C. They were harvested prior to flowering on 22 March 2017. All of the aboveground tissue was harvested, and taken to a drying room. Once dried, the tissues were ground through an Udy mill to pass a 1-mm screen on 29 March 2017. The tissue samples were stored in sealed plastic bags in cool, dark place until June when they could be used for bioassay extraction.

Experiment Design

The experimental was a 7 x 3 factorial, augmented with a control, arranged in a completely random design with three replicates. Treatments were extracts of the three cover

crops alone and in all combinations with each other (sunflower [SF], sorghum-sudangrass [SSG], winter rye [WR]; SF-WR; SSG-WR; SF-SSG; and SSG-SF-WR. There were three rates: 3%, 4%, and 5% for seven treatments: a tertiary combination, three binary combinations, and three individuals. The extract treatments were as follows: Each treatment had three replications. In addition, for all seven treatments, there was a deionized water control.

Extract preparation

Extraction methods were adapted from Javaid et al. (2006). Cover crop tissues were weighed and put into Erlenmeyer flasks with deionized water to obtain extraction concentrations of 3, 4, and 5%. The solutions were passed through cheesecloth to obtain the extract.

Experiment preparation

Petri dishes (100mm x 15mm) were lined with Schleicher & Schuell 11 cm filter paper and one hundred *Digitaria* spp. seeds were added. Three milliliters of extract were added to each petri dish. For the individual treatment extracts, 3 mL of the respective extract treatment was applied to the petri dish filter paper. For the binary treatment extracts, 1.5 of each extract was added to the petri dish filter paper. For the tertiary combination treatment extracts, 1 mL of each extract was added to the petri dish filter paper. Each petri dish was sealed with para-film so that extracts would not evaporate. Petri dishes were arranged under a windowsill and received natural light. The three replications were arranged in stacks by their treatment and concentration of each extract. Data was collected 10 days after extracts were applied to petri dishes.

Statistical Analysis

The percentage of *Digitaria* spp. seeds that germinated in each petri dish was transformed

to arcsine (square root of *x*) units before analysis. Transformed values were analyzed with analysis of variance using the GLIMMIX procedure of SAS/STAT 14.1 software (SAS Institute, Inc., 2015).

Results

Table 1. indicates that there was significant variation associated with the sources, extract, rate, and the interaction of the two, suggesting that the treatments responded differently to all the different concentrations.

For the 3% concentration rate, WR was more effective in reducing germination compared to any of the extract types alone or in any binary or tertiary extract combinations (Table 2). *Digitaria* spp. seeds treated with the individual WR treatment had a percent germination of 0.84%; whereas, SS-SF-WR combination caused a germination rate of 5.8%. In terms ranking the efficacy of germination rate reduction, the tertiary combination had equally as effective reduction of *Digitaria* spp. seed germination as the SF-WR treatment, 5.78 and 5.41%, respectively. The tertiary combination was more effective at reducing germination than were the SF, SF-SS, WR-SS, and SS extract treatments.

For the 4% concentration rate, SF-WR and WR were more effective in reducing germination compared to the tertiary combination (Table 3). These treatments had germination rates of 0.11, 0, and 0.45%, respectively. SS-SF-WR was more effective at reducing germination compared to the SF, WR-SS, SF-SS, and SS extract treatments.

For the 5% concentration rate, the tertiary combination was among the most successful at reducing the seed germination of *Digitaria* spp. (Table 4). Its reduction of germination was on

par with the SF-WR and SF-WR treatments; they all led to germination rates of 0%. SS-SF-WR was more effective compared to the treatments: SF-SS, SS, SF, and WR-SS.

Discussion

The extract concentration rates of 4 and 5% of WR were both associated with complete failure of the *Digitaria* spp. seeds to germinate. This is consistent with a study done by Barnes (1981) that found that a crop of winter rye reduced common lambsquarters (*Chenopodium album*) by 98% and common ragweed (*Ambrosia artemisiifolia*) by 90%. Another study done by Barnes (1983) reported that in the presence of winter rye, weed biomass was reduced by 93% compared to the control.

These low germination rates seen by WR extracts are also significantly similar to the SF-WR combination at rates 4% and 5%, which also led to complete failure of *Digitaria* spp. seed germination. In another study done by Mohammadi et al. 2016, winter rye extracts were applied to different weed seed species including *Sertaria viridis*, *Amaranthus retroflexus*, *Chenopodium album*, *Echinochloa curs-galli*, and *Xanthium strumarium* at the rate of 10% (100g/L H_2O). All weed seeds of all species failed to germinate (0% germination rate); whereas, in the control the rates of germination were significantly different ranging from 24.06-32.22%. Tabaglio et al. 2008 studied the effects of WR mulches on the weed species *Abutilon theophrasti* Medicus, *Amaranthus retroflexus* L., *Chenopodium album* L., and *Portulaca oleracea* L in greenhouse pots. The authors found that the mulches inhibited *Amaranthus retroflexus* germination by 40%-52% and *Chenopodium album* by 40% to 74%. Mulches were not found to have a significant effect on *Abutilon theophrasti* Medicus or *Portulaca oleracea* L.

These results are also similar to work done by Mulbeen et al. (2015) that suggests that synergism among allelopathic extracts is possible. This study found that sorghum and sunflower extracts used together are significantly more effective at reducing weed seed germination for certain weeds compared to when the extracts are used separately. For *Dactyloctenium aegyptium* weed seeds, the sorghum extract led a 76.00% germination rate and the sunflower extract led to a 76.67% germination rate. When the two aqueous extracts were used in combination, the germination rate of *Dactyloctenium aegyptium* was 67.33. This reduction in germination was significant compared to both of the germination rates of sorghum and sunflower extracts used independently. In another study, in which sunflower extracts were prepared at a higher and lower concentration, the extracts were applied to a field to reduce the presence of *Chenopodium album* (Anjum and Bajwa 2007). On a dry weight basis, the higher and lower rates of the aqueous extract reduced the weed's biomass by 75.9% and 93.5%, respectively. In terms of weed counts, the less concentrated spray led to a reduction of Chenopodium album from 24 to 11, and the stock solution spray led to a reduction of 24 to 8. Authors Bogatek et al. 2006 studied the effects on germination rate of SF aqueous extracts on wild mustard seed in laboratory bioassays. They found that 10% concentration applications had the most significant effect on weed seeds, reducing the germination rate to 15%. The control group, which experienced no application of SF aqueous extract, had a germination rate of 95-100%. The authors did include rates of 2.5% and 5%; however, the impacts on the germination rates were not as significant.

Although no studies were found to combine the allelopathic potential of SF and WR allelochemicals, studies using them individually support the findings that using them together results in very high reduction of weed seed germination. However, this study suggests, where others do not, that much lower rates of the aqueous extracts can be effective at reducing the

germination rate of weed seeds.

Experiment 2: Use of sunflower, sorghum-sudangrass, and buckwheat allelopathic extracts in laboratory bioassays

Methods and Materials

Tissue preparation

Sunflower (common oilseed), sorghum-sudangrass, and buckwheat seeds, whose cultivars were not specified, were obtained and sowed into pots size 900 with 3B SunGro soilless media on 28 September 2017. Cover crops were allowed to grow under supplemental lighting conditions (18 hours of light). They also received daily watering and received six applications of 20-10-20 NPK fertilizer at 100 ppm on 10 October 2017, 27 October 2017, 3 November 2017, 17 November 2017, 24 November 2017, and 1 December 2017. On the same dates, all plants received Peter's S.T.E.M. (Soluble Trace Element Mix). The micronutrient blend provided sulfur (2.10 ppm), boron (0.20 ppm), copper (0.48 ppm), iron (1.13 ppm), manganese (1.2 ppm), and molybdenum (0.006 ppm). The temperature set-points in the greenhouse were 18.3℃ and 23.8℃. Prior to flowering, the aboveground tissues were harvested on 10 December 2017. These were taken to a drying room. The dried tissues were ground through an Udy mill to pass a 1-mm screen on 20 December 2017. The tissue samples were stored in sealed plastic bags until 25 January 2018 in the dark at 40˚F when they could be used for bioassay extraction.

Experiment Design

The experimental was a 7 x 3 factorial, augmented with a control, arranged in a completely random design with three replicates. Treatments were extracts of the three cover crops alone and in all combinations with each other (sunflower [SF], sorghum-sudangrass [SS],

buckwheat [BW]; SF-BW; SS-BW; SF-SS; and SS-SF-BW. Each of these had three rates: 3%, 4%, and 5% for seven treatments: a tertiary combination, three binary combinations, and three individual extracts (Tables 6 and 7). Each treatment had three replicates. In addition, there was a deionized water control, which also had three replicates.

Extract preparation

Crop tissues were weighed and put into Erlenmeyer flasks with deionized water to obtain extraction concentrations of 3, 4, and 5%. The solutions were centrifuged at 5000 rpm for 30 minutes and then passed through vacuum micropore filtration (0.2 μM) to obtain each extract at its respective concentration.

Experiment preparation

Petri dishes (100mm × 15mm) were lined with Schleicher & Schuell 11 cm filter paper and one hundred *Setari faberi* seeds were added. Three mL total of extract were added to each petri dish. For the single treatment extracts, 3 mL of the respective extract treatment was applied to the petri dish filter paper. For the binary treatment extracts, 1.5 mL of each extract was added to the petri dish filter paper. For the tertiary treatment extracts, 1 mL of each extract was added to the petri dish filter paper. Each petri dish was sealed with para-film so that extracts would not evaporate. Petri dishes were arranged in a completely randomized design in a growth chamber. Petri dishes are set in a growth chamber whose relative humidity is 65% and is 22˚C; they received 16 hours of light.

Data collection

Percentage germination data were collected for the first 7 days after extracts were first applied, and again at day 14.

Statistical Analysis

Data were analyzed by using a repeated-measures analysis of variance with the GLIMMIX procedure of SAS v9.4 (SAS, Cary, NC.). Days were considered the repeated measure. The first set of analyses were conducted for all combinations (species and extract rates) plus the control as treatments (22 total). The second set of analyses considered the factorial structure of the data to determine differences for the species averaged across extract rates, and for the extract rates averaged across species. When significant ($p < 0.05$) effects were observed for germination percentages, Fisher's Least Significant Difference Test ($\alpha = 0.05$) was used to separate treatment means. To determine treatment effects on germination rate, germination percentages were regression across days of observation using the GLM procedure of SAS. Slope differences were separated using Fisher's LSD to compare all treatments with each other, and Dunnett's test to compare all treatments to the control.

Results

Overall, the germination percentage for treatment, day, and the interaction of treatment × day were significant ($p < 0.0001$ (Table 8). The treatment that caused the biggest reduction in germination at the end of the experiment was SSG-5 (Table 9). However, the following treatments were not significantly different from SSG-5: BW-4, SF-BW-4, SF-SSG-5, BW-3, SF-4, BW-SSG-4, SF-5. In addition to imparting the biggest reduction to how many *Setari faberi* seeds germinated overall, the SSG-5 treatment also represents the extract that had the most dramatic reduction in germination rate (Table 10). SSG-5's germination rate, however, was not statistically different from the following treatments: SF-BW-4, BW-4, SF-SSG-5, SF-4, SF-5, BW-3, BW-SSG-4, BW-SSG-5, BW-SSG-3, SF-SSG-4, SF-BW-5, or SF-3. Other variables of

importance were the concentration of the extract and the combination of the species used together. Using the Dunnet's test to compare how the various concentrations differed from the control, it was found that concentration 3 (3% extract) was not statistically different from the control (Table 11). It was also found that 4% and 5% extracts were statistically different from the control in that these lowered the germination rate more effectively than the control group. Concentrations 4 and 5 were not statistically different from each other in terms of reduction of germination rate. To compare the effect of combination of different species on the rate of germination, the Dunnet's test was implemented again. The tertiary combination group (SF-BW-SSG) did not differ statistically from the control (Table 12). The binary combination group and the individual extracts led to a statistically significant reduction in germination rate compared to the control. On the other hand, according to the LSD test, the tertiary combination was significantly different from the control group (Table 13). The LSD test also shows that the combinations were not significantly different among themselves.

Discussion

In terms of final germination counts of *S. faberi*, it was found that SSG-5 was the most effective in reducing the number of seeds that germinate and the rate of their germination within the 14-day period (Table 9 and Table 10). It is important to study the overall impact that allelopathic extracts have on the germination percentage in order to shed light onto what overall impact the extracts can potentially have in reducing the number of field weeds that would otherwise be able to compete with crops plants for sunlight and nutrients. These results are consistent a study done by Cheema et al. 1997 who applied extract sprays to cropping systems. Specifically, a water extract of sorghum residue called sorgaab is very common in use in

Pakistan. Cheema et al. (1997) found, upon application of sorgaab, that weed dry weight fell by 87%. Alsaadawi and Franck (2009) reviewed several publications that demonstrated that the reduction of weed biomass and weed density by sorgaab in fields of wheat (*Triticum aestivum*), maize (*Zea mays*), mung bean (*Vigna radiate*), etc. led to a significant increase in yield in the desired crops. They also note that in all of these studies that the use this aqueous extract was less costly to use compared to the alternatives of hand weeding or applying synthetic herbicides.

In addition, to overall reductions how many *Setari faberi* seeds germinated by day 14, it is also important to study the rate at which the weed seeds germinated. This is important because it relates to the importance of delaying weed establishment in the field. If weeds' germination and establishment are delayed, then this provides a window of time for the crop plants in the field to establish themselves and outcompete the weeds for sunlight. Data from the present study show that the higher concentrations of extract, 4% and 5%, resulted in significantly greater reductions in germination rate of *Setari faberi* compared to the 3% extract as well as the control (Table 11).

Work done by Allolli and Narayanareddy (1999) also shows that there is a significant negative correlation between germination of targeted seeds and concentration of extracts used. Their study employs *Eucalyptus* leaf extract that reduced germination at a significantly greater level at 10% compared to lower concentrations of 1.0, 2.5, and 5.0%. Research performed by Kara (2016) shows similar trends in that the dose of leaf extract applied is important for greater reductions in seed germination. In the study using leaf extracts of *Lavandula angustofolia* M., there was a significant negative correlation associated with *Zea mays* germination as concentration of leaf extracts increased. Another study done by Gill and Sandhu (1995) also showed a concentration dependent effect. As concentration of their sunflower extracts used

increased, the germination of pearlmillet (*Pennisetum glaucum*) decreased in laboratory bioassays. According to the authors, the degree to which the seed germination was inhibited directly correlated with the extract's concentration.

With regards to the combinations employed in this study, there was an individual extract that employed only one type of extract, as well as binary and tertiary combinations. Although the tertiary combination was not significantly different from the control, the individual and binary was different from the control. Some binary combinations were effective at reducing germination of *S. faberi* seeds (Table 12). These results show that some combinations of two different types of allelopathic extracts is significant compared to control. This is somewhat incongruent with a study conducted by Mulbeen et al. (2012). While the study showed that both the sole application of extracts and the binary application of sunflower and sorghum extracts were both significant. However, the binary application that implements equal parts sunflower and sorghum aqueous extract is statistically significant in reducing overall germination in *Trianthema portulacastrum*. Another study, conducted by Kamran et al. (2016) shows that it is more effective to combine various allelopathic extracts (3% w/v) into one natural herbicide-like spray to combat weed pressures. This work showed that maximum yield was obtained through application sorghum and moringa aqueous extracts on maize fields. They observed a 35% grain increase compare to the control group.

Conclusion

Data from the first experiment suggest that the combination of winter rye and sunflower aqueous extracts exhibit a synergistic effect on reducing *Digitaria* spp. seed germination at the rates of 4% and 5%.

The data from the second experiment show that there are synergistic effects on reduction of germination rate of *S. faberi* in combining certain allelopathic water extracts, specifically a binary combination of two residues. In addition, higher concentrations of these extracts are desirable for the greatest reduction in germination rate; concentrations 4% and 5% lowered germination rate of the weed seeds below that of the control.

It is important to consider both the overall germination recorded at the final day of the experiment and the rate at which the *Setari faberi* seeds germinated throughout the course of the experiment. More research may be done to compare the extracts used in these studies by making their experimental designs more congruent with each other.

These data shed light on how many weeds might establish in a field setting and how the allelopathic extracts might delay the weeds' establishments. However, laboratory bioassays are preliminary studies that should not wholly suggest an allelopathic extract's ability to reduce weed pressure in the field. Therefore, additional research must be done to examine if these cover crop extracts can be used additively in the field to reduce weed seed emergence and biomass in a setting that has a large seed bank. In addition, only one type of weed seed was tested in each experiment. It is possible that these allelochemicals may have different synergistic impacts in the face of other species of weed seeds found in the seed bank.

Experiment 1 Tables

Table 1. Source effects for analysis of variance

Type III Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
extract	6	62	23.44	<.0001
rate	2	62	104.03	<.0001
extract*rate	12	62	5.32	<.0001

Table 2: T Grouping for extract*rate interaction for 3% extracts

T Grouping for extract*rate Least Squares Means Slice (Alpha=0.05)					
LS-means with the same letter are not significantly different.					
Slice	**extract**	**Arcsin Estimate**	**Arcsin converted to %**		
rate 3	**SS**	0.4434	19.5306		A
rate 3	**WR-SS**	0.3954	15.5719	B	A
rate 3	**SF-SS**	0.3824	14.573	B	A
rate 3	**SF**	0.3431	11.7431	B	
rate 3	**WR-SS-SF**	0.2404	5.7752		C
rate 3	**SF-WR**	0.2328	5.4184		C
rate 3	**WR**	0.09142	0.8357		D

Table 3: T Grouping for extract*rate interaction for 4% extracts

T Grouping for extract*rate Least Squares Means Slice (Alpha=0.05)					
LS-means with the same letter are not significantly different.					
Slice	**extract**	**Arcsin Estimate**	**Arcsin converted to %**		
rate 4	**SS**	0.4064	0.1115		A
rate 4	**SF-SS**	0.1494	2.2332		B
rate 4	**WR-SS**	0.1429	2.0415		B
rate 4	**SF**	0.1419	2.0133		B
rate 4	**WR-SS-SF**	0.06678	0.4459	C	B
rate 4	**SF-WR**	0.03339	0.1115	C	
rate 4	**WR**	-1.84E-16	0	C	

Table 4: T Grouping for extract*rate interaction for 5% extracts

T Grouping for extract*rate Least Squares Means Slice (Alpha=0.05)					
LS-means with the same letter are not significantly different.					
Slice	**extract**	**Arcsin Estimate**	**Arcsin converted to %**		
rate 5	**WR-SS**	0.183	3.3482		A
rate 5	**SF**	0.1159	1.3427	B	A
rate 5	**SS**	0.03339	0.1115	B	C
rate 5	**SF-SS**	0.03339	0.1115	B	C
rate 5	**WR-SS-SF**	2.52E-15	0		C
rate 5	**SF-WR**	-6.60E-17	0		C
rate 5	**WR**	-2.26E-16	0		C

Table 5: Values converts to percentage values using ArcSin. The convert value indicates the rate of germination (%) of *Digitaria* spp. seed.

extract	**rate**	**Arcsin Estimate**	**Arcsin converted to %**
Control	0	0.71881	49.4007
SF	3	0.34308	11.7431
SF	4	0.1419	2.0133
SF	5	0.11588	1.3427
SF-SS	3	0.38243	14.573
SF-SS	4	0.14944	2.2332
SF-SS	5	0.03339	0.1115
SF-WR	3	0.23283	5.4184
SF-WR	4	0.03339	0.1115
SF-WR	5	0	0
SS	3	0.44336	19.5306
SS	4	0.40645	16.445
SS	5	0.03339	0.1115
WR	3	0.09142	0.8357
WR	4	0	0
WR	5	0	0
WR-SS	3	0.39542	15.5719
WR-SS	4	0.14288	2.0415
WR-SS	5	0.183	3.3482
WR-SS-SF	3	0.24038	5.7752
WR-SS-SF	4	0.06678	0.4459
WR-SS-SF	5	0	0

Experiment 2 tables

Table 6. Cover crops and their respective abbreviations treatment names

Cover crops	Sunflower	Buckwheat	Sorghum-sudangrass
Abbreviations	SF	BW	SSG

Table 7. Treatments in individual, binary, and tertiary combinations at their respective concentrations

Individual treatments at 3% concentration	SSG-3
	SF-3
	BW-3
Individual treatments at 4% concentration	SSG-4
	SF-4
	BW-4
Individual treatments at 5% concentration	SSG-5
	SF-5
	BW-5
Binary treatments at 3% concentration	SSG-SF-3
	SSG-BW-3
	SF-BW-3
Binary treatments at 4% concentration	SSG-SF-4
	SSG-BW-4
	SF-BW-4
Binary treatments at 5% concentration	SSG-SF-5
	SSG-BW-5
	SF-BW-5
Tertiary treatmnet at 3% conc.	SSG-SF-BW-3
Tertiary treatmnet at 4% conc.	SSG-SF-BW-4
Tertiary treatmnet at 5% conc.	SSG-SF-BW-5

Table 8. Source effects for analysis of variance.

Source	**DF**	**Type I SS**	**Mean Square**	**F Value**	**Pr > F**
day	1	118834.0420	118834.0420	1388.66	<.0001
treat	21	16231.8971	772.9475	9.03	<.0001
day*treat	21	5350.4689	254.7842	2.98	<.0001

Table 9: Percentage germination at day 14 for all treatments. Means followed by the same letter are not different according to Fisher's LSD (α=0.05).

LS-means with the same letter are not significantly different.						
Slice	**treat**	**Estimate**				
day 14	**Control**	75.8718			A	
day 14	**SF-SSG-3**	69.0000	B		A	
day 14	**SSG-3**	67.0000	B		A	
day 14	**SSG-4**	64.0000	B		A	C
day 14	**SF-BW-3**	62.6667	B		A	C
day 14	**SF-BW-SSG-5**	61.0000	B	D	A	C
day 14	**BW-5**	57.3333	B	D		C
day 14	**SF-BW-SSG-3**	55.0000	B	D	E	C
day 14	**SF-BW-SSG-4**	53.0000		D	E	C
day 14	**SF-3**	52.0000		D	E	C
day 14	**SF-BW-5**	51.5000		D	E	C
day 14	**SF-SSG-4**	50.6667		D	E	
day 14	**BW-SSG-5**	48.0000	F	D	E	
day 14	**BW-SSG-3**	47.3333	F	D	E	
day 14	**SF-5**	47.0000	F	D	E	G
day 14	**BW-SSG-4**	46.6667	F	D	E	G
day 14	**SF-4**	45.0000	F	D	E	G
day 14	**BW-3**	44.5000	F	D	E	G
day 14	**SF-SSG-5**	42.3333	F		E	G
day 14	**SF-BW-4**	38.0000	F		E	G
day 14	**BW-4**	36.0000	F			G
day 14	**SSG-5**	34.6667				G
The LINES display does not reflect all significant comparisons. The following additional pairs are significantly different: (SF-SSG-3 14,BW-5 14), (BW-5 14,SF-4 14), (BW-5 14,BW-3 14), (SF-BW-SSG-4 14,SF-BW-4 14), (BW-SSG-4 14,SSG-5 14).						

Table 10: Comparison of germination rate slopes of each extract treatment at its given concentration

Means with the same letter are not significantly different.									
t Grouping							**Mean**	**N**	**group**
			A				6.7130	14	Control
	B		A				5.9899	24	SF-SSG-3
	B		A		C		5.8420	24	SSG-3
	B	D	A		C		5.5462	24	SF-BW-3
E	B	D	A		C		5.4329	16	SSG-4
E	B	D	F		C		5.0519	24	BW-5
E	B	D	F		C		5.0238	8	SF-BW-SSG-5
E	B	D	F		C	G	4.6602	8	SF-BW-SSG-3
E	B	D	F		C	G	4.5963	16	SF-BW-SSG-4
E	B	D	F	H	C	G	4.5736	16	SF-3
E	B	D	F	H	C	G	4.4665	16	SF-BW-5
E	B	D	F	H	C	G	4.4033	24	SF-SSG-4
E		D	F	H	C	G	4.1905	24	BW-SSG-3
E		D	F	H		G	4.1255	16	BW-SSG-5
E		D	F	H		G	4.0563	24	BW-SSG-4
E		D	F	H		G	4.0444	16	BW-3
E		D	F	H		G	3.9037	16	SF-5
E			F	H		G	3.8788	24	SF-4
			F	H		G	3.4978	24	SF-SSG-5
				H		G	3.2807	24	BW-4
				H		G	3.2673	16	SF-BW-4
				H			2.9271	24	SSG-5

Table 11: Dunnett's-test of concentration compared to control for germination rates

Comparisons significant at the 0.05 level are indicated by *.**				
group Comparison	**Difference Between Means**	**Simultaneous 95% Confidence Limits**		
3* - 0	-1.6188	-3.4884	0.2509	
4* - 0	-2.6324	-4.4986	-0.7662	***
5* - 0	-2.6851	-4.5586	-0.8116	***

* 3, 4, and 5 refer to 3%, 4%, and 5% concentrations of allelopathic extracts, respectively

Table 12: Dunnett's-for combination compared to control for germination rates

Comparisons significant at the 0.05 level are indicated by *.**				
group Comparison	**Difference Between Means**	**Simultaneous 95% Confidence Limits**		
3* – 0	-1.9938	-4.2117	0.2241	
2* – 0	-2.2642	-4.2576	-0.2708	***
1* – 0	-2.4151	-4.4106	-0.4197	***

* 3, 2, and 1 refer to the tertiary, binary, and individual combinations respectively

Table 13: LSD - comparisons among all combination treatments

Comparisons significant at the 0.05 level are indicated by *.**				
group Comparison	**Difference Between Means**	**95% Confidence Limits**		
0 - 3	1.9938	0.0078	3.9799	***
0 - 2	2.2642	0.4792	4.0492	***
0 - 1	2.4151	0.6283	4.2020	***
3 - 0	-1.9938	-3.9799	-0.0078	***
3 - 2	0.2704	-0.7599	1.3006	
3 - 1	0.4213	-0.6122	1.4548	
2 - 0	-2.2642	-4.0492	-0.4792	***
2 - 3	-0.2704	-1.3006	0.7599	
2 - 1	0.1509	-0.4057	0.7076	
1 - 0	-2.4151	-4.2020	-0.6283	***
1 - 3	-0.4213	-1.4548	0.6122	
1 - 2	-0.1509	-0.7076	0.4057	

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